TechNotes

HyTest

Clinical and Research Area | Fertility and Pregnancy | Cardiac Markers

Pregnancy-associated plasma protein-A (PAPP-A)

Pregnancy-associated plasma protein-A (PAPP-A) is a metalloprotease that belongs to the metzincin superfamily of zinc peptidases. Its main substrate is the insulin-like growth factor binding protein-4 (IGFBP-4). This cleavage causes the release of bound IGF, which plays an important role in promoting cell differentiation and proliferation. PAPP-A was first identified in the serum of pregnant women, hence its name. It was later shown to be expressed in multiple tissues.

Two forms of PAPP-A

Heterotetrameric PAPP-A (htPAPP-A) is a screening marker for Down syndrome. htPAPP-A levels in maternal serum increases with gestational age until the full-term. If the concentration of htPAPP-A in the first trimester is markedly decreased, this indicates a higher risk of Down syndrome (1). htPAPP-A is a protein complex that consists of two PAPP-A subunits and two proforms of eosinophil basic proteins (proMBP) that are covalently linked to each other. proMBP has been shown to inhibit the protease activity of PAPP-A in this heterotetrameric complex (2).

Homodimeric PAPP-A (dPAPP-A) is abundantly expressed in unstable coronary atherosclerotic plaques (3). dPAPP-A circulates as a homodimer and not in a complex with proMBP. Based on several studies, dPAPP-A has been considered to be a promising marker of plaque destabilization in patients with acute coronary syndrome (ACS). Unfortunately, dPAPP-A assays have been shown to also detect htPAPP-A, which is the Down syndrome marker that is not related to atherosclerotic plaques. To prevent this, a dPAPP-A assay should be designed to only recognize dPAPP-A and not cross-react with htPAPP-A.

Another limitation to the use of dPAPP-A as a cardiac marker is the fact that the measurements were shown to be affected by heparin, which is an anticoagulation agent often used as part of the treatment procedure with patients suffering from acute myocardial infarction. Therefore, to use dPAPP-A as a cardiac biomarker, the heparin injections should be taken into account when analyzing the samples. A promising surrogate marker for dPAPP-A is its main substrate, IGFBP-4. For more information, please see our IGFBP-4 TechNotes.

CLINICAL UTILITY

- ✓ First trimester screening marker for Down syndrome
- ✓ Marker of atherosclerotic plaque destabilization

Reagents for immunoassay development

We provide monoclonal antibodies (MAbs) that are specific to PAPP-A and proMBP that allow for the development of highly sensitive, quantitative htPAPP-A immunoassays. We also provide reagents for the development of the dPAPP-A specific assay. In addition, we provide the dimeric recombinant human PAPP-A antigen to be used in PAPP-A assays.

Monoclonal antibodies specific to htPAPP-A

We provide several different MAbs that are specific to htPAPP-A. Some of the MAbs recognize the PAPP-A subunit while some are specific to the proMBP part of the heterotetrameric complex.

Total PAPP-A and htPAPP-A sandwich immunoassays

All MAbs were tested in pairs in sandwich fluoroimmunoassays as capture and detection antibodies with both forms of the antigen – htPAPP-A and dPAPP-A. The antibody pairs that perform best in our in-house assays are listed in Table 1. Calibration curves for two suggested pairs are shown in Figure 1.

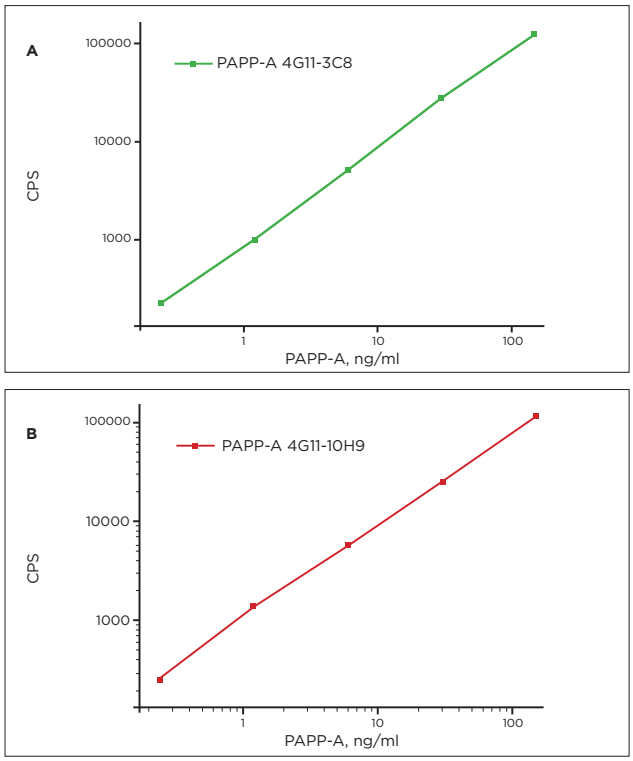

Figure 1. Calibration curves for two PAPP-A sandwich immunoassays. (A) 4G11 – 3C8 and (B) 4G11 – 10H9.
Capture MAb: 4G11 (biotinylated)
Detection MAbs: 3C8 or 10H9 (labeled with stable Eu^{3+} -chelate)
Antigen: native htPAPP-A
Mixture of antibodies and antigen was incubated for 30 minutes at room temperature in streptavidin-coated plates.

Table 1. Recommended pairs for htPAPP-A and total PAPP-A sandwich immunoassay.

Detection of human htPAPP-A antigen (capture – detection)	Detection of total PAPP-A (htPAPP-A and/or dPAPP-A) (capture – detection)
10E2cc - 5H9	10E2cc - 10E1cc
5H9 - 10E2cc	4G11 - 3C8
5H9 - 7A6	4G11 - 10H9
10E1cc - 11E4	10E1cc - 7A6

Monoclonal antibodies specific to dPAPP-A

We offer a few MAbs that only recognize dPAPP-A and do not cross-react with htPAPP-A.

Selective dPAPP-A sandwich immunoassay

The antibody pair PAPP52-PAPP30 specifically recognizes dPAPP-A. In this prototype assay, one MAb is specific to dPAPP-A (Cat.# 4PD4), while the other MAb recognizes all known forms of PAPP-A (Cat.# 4P41). This prototype assay was tested with dPAPP-A purified from atherosclerotic coronary arteries, as well as with purified native htPAPP-A and human recombinant wild-type dPAPP-A (in-house preparation). The assay was able to recognize dimeric forms of the antigen with high specificity and with negligible cross-reactivity (< 1 %) with htPAPP-A. This MAb combination could be used for the development of a highly sensitive sandwich immunoassay that is suitable for the selective quantitative measurements of dPAPP-A in human blood.

dPAPP-A levels in the blood of patients with ACS

We measured the concentration of dPAPP-A in the plasma obtained from 43 patients with ACS (acute myocardial infarction, unstable angina) using the prototype assay PAPP52-PAPP30. The samples were withdrawn 3-20 hours following the onset of chest pain. As a control, we used plasma samples obtained from 34 non-ACS patients. The dPAPP-A levels in plasma from ACS patients were 2.77-fold higher than the plasma of the control group ($P<0.0005$) (Figure 2).

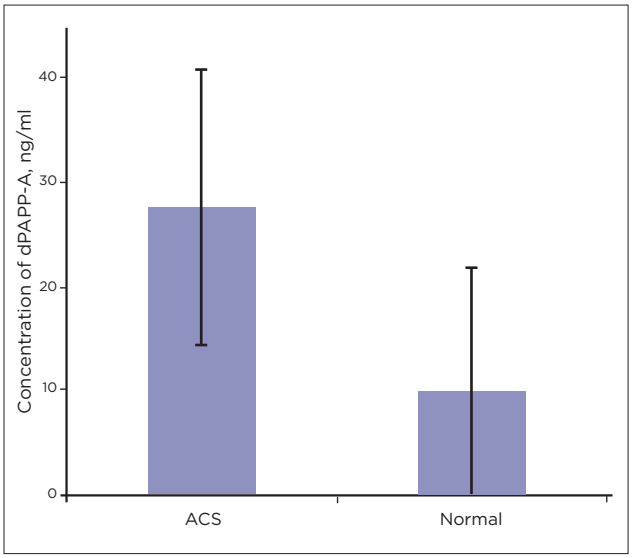

Figure 2. dPAPP-A concentration in plasma samples of 43 ACS patients (ACS) and 34 non-ACS patients control group (Normal) measured by PAPP52 - PAPP30 sandwich immunoassay (mean+/-SD).
Capture MAb: PAPP52
Detection MAb: PAPP30 (labeled with Eu^{3+} chelate)
Incubation volume: 100 µl.
Incubation time: 30 min at room temperature.

Dimeric recombinant human PAPP-A

Our dimeric recombinant human PAPP-A has E483A mutation for stabilization of the protein due to the suppression of proteolytic activity and autocleavage. The dimeric recombinant PAPP-A antigen contains His10-tag and is expressed in mammalian cells. The product is purified by metal affinity chromatography. The protein presentation with 5% sucrose is optimized for storage in lyophilized form. The purity of the protein is >90% (Figure 3).

Recombinant human dimeric PAPP-A is immuno-chemically active in different sandwich immunoassay pairs, (Figure 4).

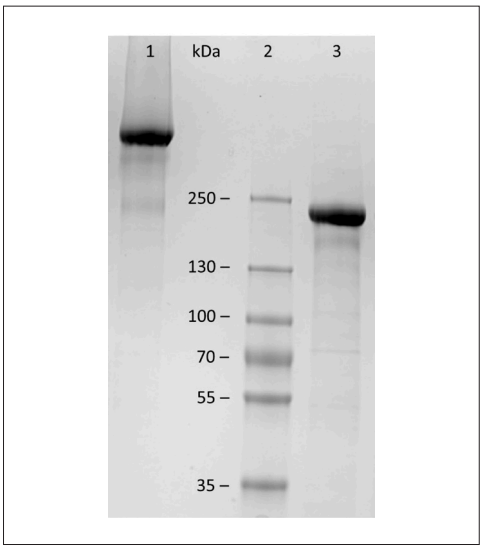

Figure 3. SDS-PAGE of recombinant human dimeric PAPP-A.
1. in non-reducing conditions, 5 µg;
2. molecular weight markers;
3. in reducing conditions, 5 µg.

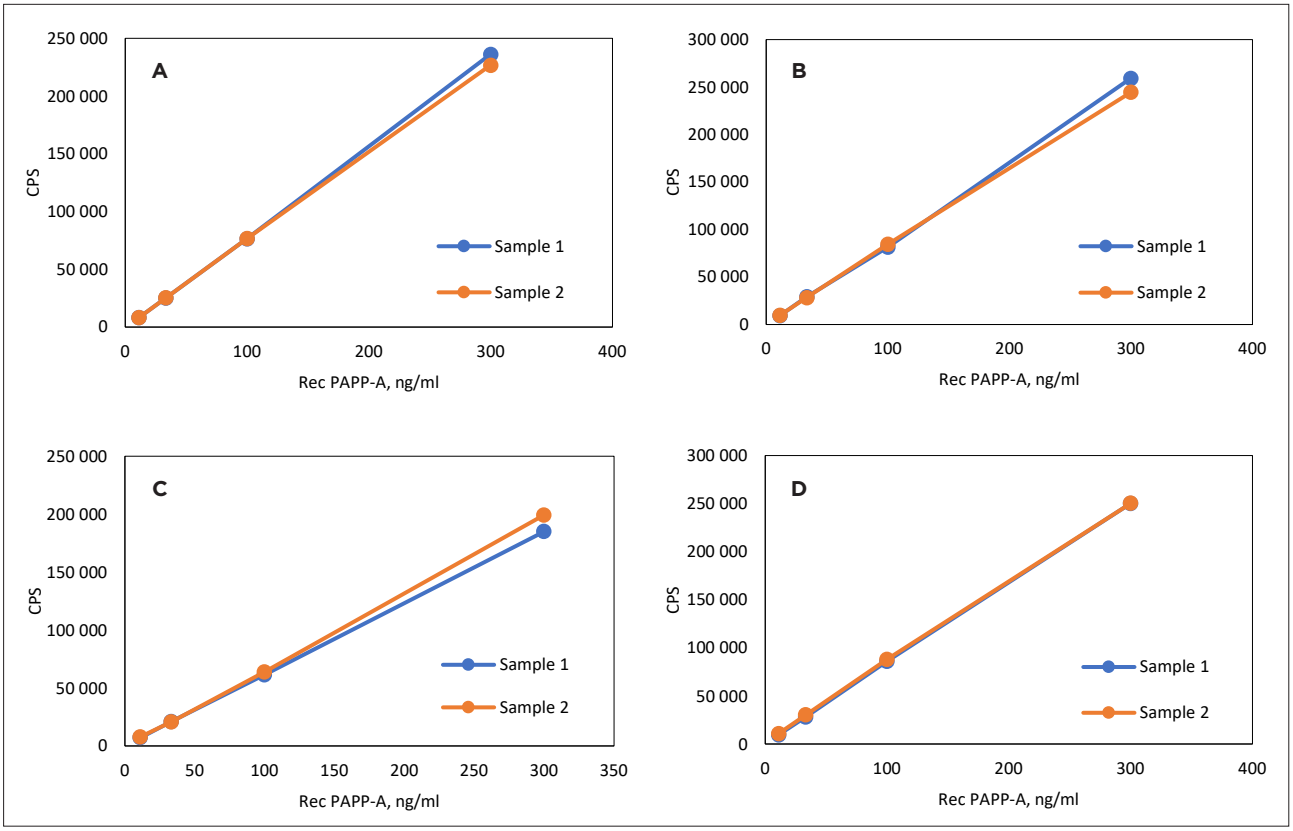

Figure 4. Calibration curve for the recombinant human dimeric PAPP-A. With A) 10A5-3C8, B) 10E2cc-10E1cc, C) 4G11-10H9, and D) 10E1cc-7A6 (capture-detection) sandwich immunoassays. The capture antibodies were adsorbed on the immunoassay microplates. A mixture of antigen and detection antibodies labelled with Eu^{3+} stable chelate was incubated for 30 minutes at room temperature. Samples 1 and 2 were independently produced recombinant human dimeric PAPP-A antigen samples.

PAPP-A immunodetection in Western blotting

The MAbs 3C8 and 7A6 recognize the PAPP-A subunit whereas the MAbs 5H9 and 11E4 recognize the proMBP subunit of htPAPP-A in Western blotting after SDS-PAGE in reducing and non-reducing conditions (Figure 5). The MAbs 4G11 and 10E1cc recognize htPAPP-A in Western blotting only after electrophoresis in non-reducing conditions (data for 4G11 not shown here).

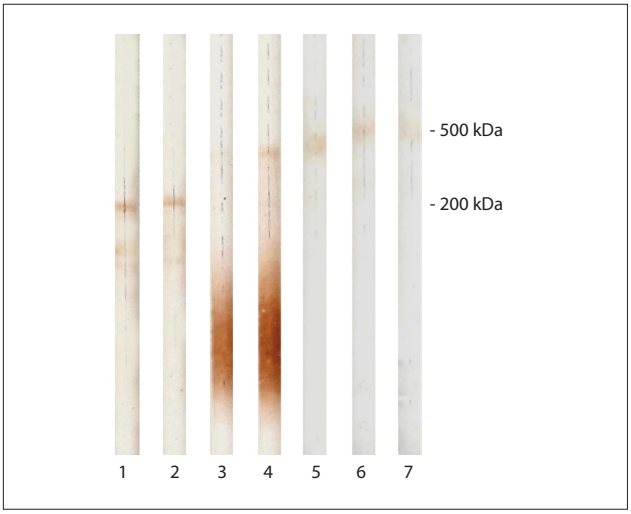

Figure 5. Detection of human PAPP-A and proMBP subunits of htPAPP-A by monoclonal antibodies in Western blotting.
Lane 1: 7A6
Lane 2: 3C8
Lane 3: 5H9 (proMBP-specific)
Lane 4: 11E4 (proMBP-specific)
Lane 5: 7A6
Lane 6: 3C8
Lane 7: 10E1
Lanes 1-4: after SDS-PAGE in reduction conditions.
Lanes 5-7: after SDS-PAGE in non-reducing conditions. The heterotetrameric complex was detected by anti-PAPP-A MAbs.

Ordering information

MONOCLONAL ANTIBODIES

Product name	Cat. #	MAb	Subclass	Remarks
Pregnancy-associated plasma protein A (PAPP-A), human	4P41cc	10E1cc	IgG2b	*In vitro*, EIA, WB, PAPP-A subunit
		10E2cc	IgG2b	*In vitro*, EIA, PAPP-A subunit
	4P41	5H9	IgG2b	EIA, proMBP subunit
		4G11	IgG2a	EIA, WB, PAPP-A subunit
		3C8	IgG2a	EIA, WB, PAPP-A subunit
		10H9	IgG2a	EIA, PAPP-A subunit
		11E4	IgG2b	WB, proMBP subunit
		7A6	IgG2a	EIA, PAPP-A subunit
		PAPP52	IgG1	EIA, PAPP-A subunit
Dimeric form of pregnancy-associated plasma protein A (dPAPP-A), human	4PD4	PAPP30	IgG1	EIA, dimeric form of PAPP-A only

ANTIGEN

Product name	Cat. #	Purity	Source
PAPP-A, human, recombinant	8PA1	>90%	Recombinant

Please note that some or all data presented in this TechNotes has been prepared using MAbs produced in vivo. MAbs produced in vitro are expected to have similar performance.

References

1. **Palomaki GE, Lambert-Messerlian GM, Canick JA** A summary analysis of Down syndrome markers in the late first trimester.// Adv Clin Chem. 2007;43:177-210.
2. **Overgaard, MT., Haaning, J., Boldt, HB., Olsen, IM., Laursen, LS., et al.** Expression of recombinant human pregnancy-associated plasma protein-A and identification of the proform of eosinophil major basic protein as its physiological inhibitor.// J Biol Chem; 275:31128-33 (2000).
3. **Bayes-Genis, A., Conover, C. A., Overgaard, M. T., Bailey, K. R., Christiansen, M., Holmes, D. R. Jr, et al.** Pregnancy-associated plasma protein A as a marker of acute coronary syndromes.// N Engl J Med, 345 (14), 1022-9 (2001).

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